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# Diphtheria–tetanus–pertussis vaccine administered simultaneously with measles vaccine is associated with increased morbidity and poor growth in girls. A randomised trial from Guinea-Bissau

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ABSTRACT

*Background:* Combined vaccination with diphtheria–tetanus–pertussis (DTP) and measles vaccine (MV) has been associated with increased mortality in observational studies. Among children missing MV and a dose of DTP and oral polio vaccine (OPV), we conducted a randomised trial of providing MV + DTP + OPV simultaneously, as currently recommended, or MV + OPV only, and examined the effect on morbidity and growth. We hypothesised that the MV + OPV group would experience less morbidity and grow better. Due to previous observations of sex differences in the non-specific effects of vaccinations, we analysed all data stratified by sex.

*Methods:* At the Bandim Health Project in Guinea-Bissau, 568 children who were due to receive MV and who were missing either DTP3 or DTP booster were enrolled in the study. A subgroup of 332 children was followed intensively to register adverse events and infections in the first month after vaccination. A subgroup of 276 children was followed every third month for a year to monitor growth. All children were followed for one year for infectious diseases, consultations, and hospitalisations.

*Results:* As expected, adverse events were more common in the MV + DTP + OPV group; diarrhoea and use of medication were increased among girls but not among boys (both $p = 0.02$, test of interaction between DTP and sex). Febrile disease with vesicular rash, as well as consultations and hospitalisations tended to be more common in the MV + DTP + OPV group than in the MV + OPV group; the hazard ratio (HR) for febrile disease with vesicular rash was 1.86 (1.00; 3.47). The strongest tendencies for more febrile diseases and hospitalisations in the MV + DTP + OPV group were found in girls. Overall, growth did not differ by randomisation group. However, results differed by sex. Girls in the MV + DTP + OPV group had a consistent pattern of worse *z*-scores for weight, height, and mid-upper-arm-circumference (MUAC) than girls in the MV + OPV group. The effect was opposite for boys, with boys in the MV + OPV group faring worse than those in the MV + DTP + OPV group, the interaction test for sex and DTP being significant for weight at 6 and 9 months, for MUAC at 12 months and for weight-for-height at 3 and 9 months after randomisation.

*Conclusion:* This is the first randomised trial of the non-specific effects of DTP and supports that these effects may be sex-differential and of clinical and anthropometric importance. Combined vaccination with DTP + MV + OPV may be detrimental for girls.



## 1. Introduction

Routine childhood vaccines protect against the specific infectious diseases for which the vaccines are provided. However, several observational studies and a few randomised trials have indicated that they may also have non-specific effects on all-cause mortality. These non-specific effects seem to be strongest for girls. Measles vaccine (MV) has beneficial effects especially

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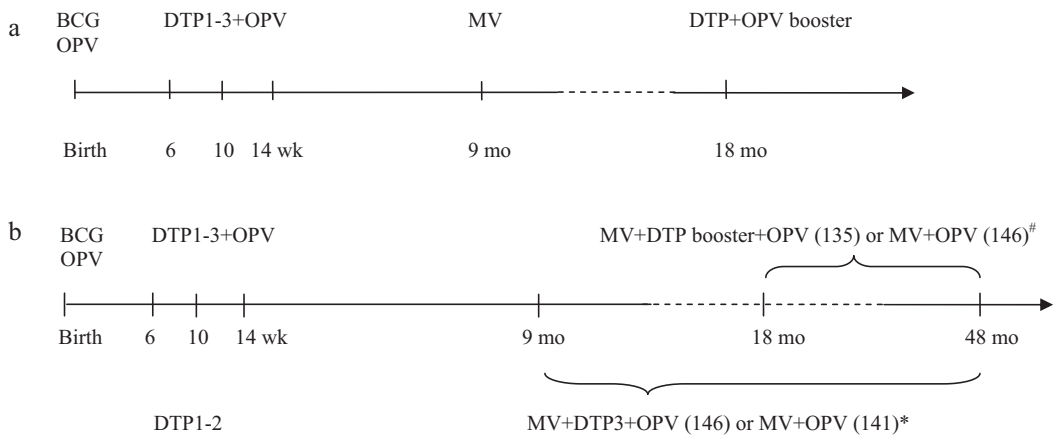


**Fig. 1.** Vaccination schedules: (a) routine-vaccination schedule, (b) randomisation schedule. *Came late for DTP3 and randomised to MV + DTP3 + OPV or MV + OPV, #Came late for MV and randomised to MV + DTP booster + OPV or MV + OPV. Previous vaccinations may have been provided until one month before randomisation. (): number of children included.

for girls [1–5] whereas diphtheria–tetanus–pertussis (DTP) vaccine has worryingly been associated with increased female mortality [2–11].

The combination of vaccines may also be important. Normally MV, recommended at 9 months of age, is given after the recommended three doses of DTP at ages 6, 10, and 14 weeks. However, vaccines are frequently delayed, particularly in resource poor settings. In particular, children who come for MV and who are missing one or more DTP vaccines are given both vaccines simultaneously. The effect of this policy on overall mortality and morbidity has never been studied. Several observational studies have indicated that receiving DTP together with MV is associated with higher mortality than receiving MV only. A study from The Gambia showed a relative risk of death of 5.59 (2.10; 14.8) for children having a third DTP (DTP3) together with MV compared with children having MV only [9]. Data from Congo showed a mortality rate ratio (MRR) of 5.38 (1.37; 21.15) [10] and from Malawi of 5.27 (1.11; 25.0) for children who received DTP together with MV compared with children who received MV only [11]. Such differences may partly be explained by selection bias, children who come late for their vaccines and receive MV and DTP simultaneously having higher mortality *a priori*. However, the effect estimates are so large that it seems implausible that they are due to selection bias alone.

To test the hypothesis that receiving DTP and MV simultaneously has negative non-specific effects compared with receiving MV only, we conducted a randomised trial among children missing both a dose of DTP and MV. The children were randomised to receive MV + DTP + OPV (usual practice) or MV + OPV. We followed children for morbidity and growth. Our hypothesis was that not providing DTP together with MV would be associated with a reduction in severe morbidity and with improved growth. Based on previous observations of sex differences in non-targeted effects we analysed all data stratified by sex.

Due to a better than expected coverage with the recommended DTP3 and MV (thus few children needing DTP and MV simultaneously) we enrolled only 568 children of the 2000 children planned to be enrolled; and due to a national vaccination campaign many children received MV *after* randomisation and had to be censored. However, this is the first randomised trial of MV + DTP + OPV vs. MV + OPV and the results have important implications for future trials of non-specific effects of vaccines and for deciding future vaccination programmes.

## 2. Methods

### 2.1. Setting

The study area consists of six districts in Bissau, the capital of Guinea-Bissau, West Africa. The Bandim Health Project (BHP) has been working in this study area for 30 years, and a health and demographic surveillance system (HDSS) has been established and functioned for all these years. The current population in the districts is around 100,000. The basis for the registration system has been mapping and numbering of all houses in the area. All houses are visited every month to register new pregnancies and births. Once a newborn is identified, the child is followed with a home visit every third month to register growth, breastfeeding practices, vaccinations, infections, hospitalisations and survival. The national hospital Simão Mendes receives the majority of admissions from the study area. Routine childhood vaccinations are provided at the three health centres in each of districts Bandim, Belem and Cuntum. The recommended vaccine schedule is shown in Fig. 1(a).

### 2.2. Study design

Eligible children were between 9 months and 48 months, had not received MV at routine vaccination, but had received at least 2 doses of DTP previously. They came for MV and for either DTP3 (9–48 months old) or DTP-booster (18–48 months old). All children are normally given an oral polio vaccine (OPV) together with DTP. Hence, we randomised them to either MV + DTP + OPV or MV + OPV (Fig. 1(b)).

MV was provided in a national campaign in May 2006. MV received in the campaign was not considered to replace the MV at routine vaccinations and children who received MV in the campaign would still receive DTP + OPV together with MV – and be eligible for the trial – if they had no MV at routine vaccinations and were 9 months or older.

Children who took part in the vaccination campaign received a "campaign card". BHP field workers monitored all teams providing MV and those children not seen by the vaccination teams were visited at home to obtain information on whether they had received MV elsewhere. The information on who had received MV was not available in a reliable form to the teams conducting the screening for eligibility and enrolment but was available for the subsequent data analyses. Children, who received MV in the campaign, also

received vitamin A, and those 1 year or older received Mebendazole in the campaign.

### 2.3. Enrolment

The children were identified in two different ways: first, children who were brought by their mothers for the routine vaccination sessions in the morning at the three local health centres were assessed for vaccination status and eligibility; and second, using the BHP database for vaccinations we identified children missing MV and DTP3 or DTP booster. The latter children were visited at home and invited to come to the health centres in the afternoon to participate in the study.

The study was explained to the mother/guardian in the local language (Crioul), written explanation in the official language (Portuguese) was provided, and they were asked to sign a consent form. At inclusion a medical history and symptoms on the day of inclusion were registered by project assistants, and weight, height, left upper-arm-circumference and temperature were measured by a nurse or an assistant. Children called for vaccination were examined by a physician for signs of infectious diseases. Children with acute febrile illness, diagnosed by the physician, or with an axillary temperature above 37.5 °C at examination were not included but treated if necessary and told to come back when they were well again. Routine vaccinations were managed by nurses and no medical examination was performed. Children with an axillary temperature above 37.5 °C were not included at routine vaccinations.

### 2.4. Randomisation

Randomisation to the MV + DTP + OPV group or MV + OPV group was done separately for boys and girls.

The mother/guardian drew a randomisation note from an envelope with 12 notes (6 notes marked "MV + DTP + OPV" and 6 notes marked "MV + OPV") – white envelopes for boys with lot numbers starting from 1000, and yellow envelopes for girls with lot numbers starting from 2000, determining the treatment allocation of the child. Notes were folded, non-transparent and stapled. Nurses vaccinated the children according to the randomisation result. For those randomised to the MV + OPV group it was written on the vaccination card that the child should not receive DTP to ensure that the child was not vaccinated with DTP during the study period.

The DTP vaccine used in Guinea-Bissau was diphtheria–tetanus–whole-cell-pertussis from the Serum Institute of India until August 2007, and subsequently from Bio Pharma in Indonesia. MV was Edmonston-Zagreb measles vaccine from Serum Institute of India.

### 2.5. Cohort

#### 2.5.1. Total cohort

Between October 25, 2005 and April 28, 2008, a total of 578 children were randomised (Fig. 2(a)). Of 292 children missing both MV and DTP3 at inclusion, 141 children were randomised to MV + DTP3 + OPV, 146 to MV + OPV and 5 children were excluded due to errors at inclusion. Of 286 children missing both MV and a DTP booster at inclusion, 146 were randomised to MV + DTP booster + OPV, 135 were randomised to MV + OPV and 5 were excluded due to error at inclusion. Thus, 568 were randomised and not excluded (287, 145 boys and 142 girls, received MV + DTP + OPV and 281, 148 boys and 133 girls, received MV + OPV) (Figs. 1(b) and 2(a)).

#### 2.5.2. Morbidity sub-study cohort

332 children who entered the study on specific week days participated in the study of adverse events ($n = 171$ in the MV + OPV group and $n = 161$ in the MV + DTP + OPV group). Logistics determined the time periods and week days for inclusion in the sub-studies (Fig. 2(b)).

#### 2.5.3. Anthropometry sub-study cohort

276 were included in the time period July 18, 2006 to November 30, 2007 ($n = 139$ in the MV + OPV group and $n = 137$ in the MV + DTP + OPV group) (Fig. 2(c)).

### 2.6. Follow-up

#### 2.6.1. Morbidity

All 568 children were followed by the HDSS at the routine three-monthly home visits at which infectious diseases easily recognized by the mother were registered (measles, whooping cough, "*cambletch*" and "*varicella*"). "Varicella" reported by a mother is likely to be chickenpox, whereas "Cambletch" might represent the bullous form of chickenpox, but also impetigo or primary herpes simplex. In previous studies, varicella-zoster-virus seroconversion was found in 105 of 127 (83%) of cases reported as "Varicella" and in 6 of 35 (18%) of cases reported as "Cambletch" [12]. Furthermore all children from the study area who were *hospitalised* at the national hospital, or who came for *consultations* at the hospital or at the three health centres in the study area, were registered. Hospitalisations were also registered at follow-up visits in the adverse morbidity events and anthropometry sub-studies.

*2.6.1.1. Adverse morbidity events sub-study.* The 332 children included in an adverse-events sub-study were followed daily for 3 days and weekly for 1 month following randomisation to register morbidity events (Fig. 2(b)). Symptoms reported by the mother (diarrhoea, vomiting, cough, loss of appetite, restlessness, crying, convulsions and other symptoms), objective signs (local reaction at vaccination spot, lesions in skin, conjunctivitis, running nose, difficulties breathing, respiratory frequency, and temperature), consultations and medication were registered by a field assistant. Medication was most often paracetamole, being one of several or the only drug taken by 84% of children who reported using medication.

#### 2.6.2. Anthropometry sub-study

The 276 children included in the anthropometry sub-study were visited by assistants every third month for 1 year to monitor growth (Fig. 2(c)). Children were weighed without clothes on a Seca digital baby scale. Length/height was measured on a Seca baby length measuring mat or, according to WHO standards, standing by a Seca Stadiometer if the child was 2 years or older. Mid-upper-arm-circumference (MUAC) was measured on the left arm by a TALC insertion tape.

### 2.7. Sample size

We expected to recruit 2000 children during a 3-year period. This sample size was based on a 3% annual mortality rate and the hypothesis of 35% reduced mortality following MV + OPV only. However, due to several outreach campaigns, the coverage of the recommended vaccines soon became much higher than expected and fewer children than expected lacked both MV and DTP. Furthermore, many children received MV after inclusion due to the measles vaccination campaign in May 2006, and were therefore censored after randomisation. In 2008 Guinea-Bissau decided to change from DTP to pentavalent vaccine (DTP + hepatitis

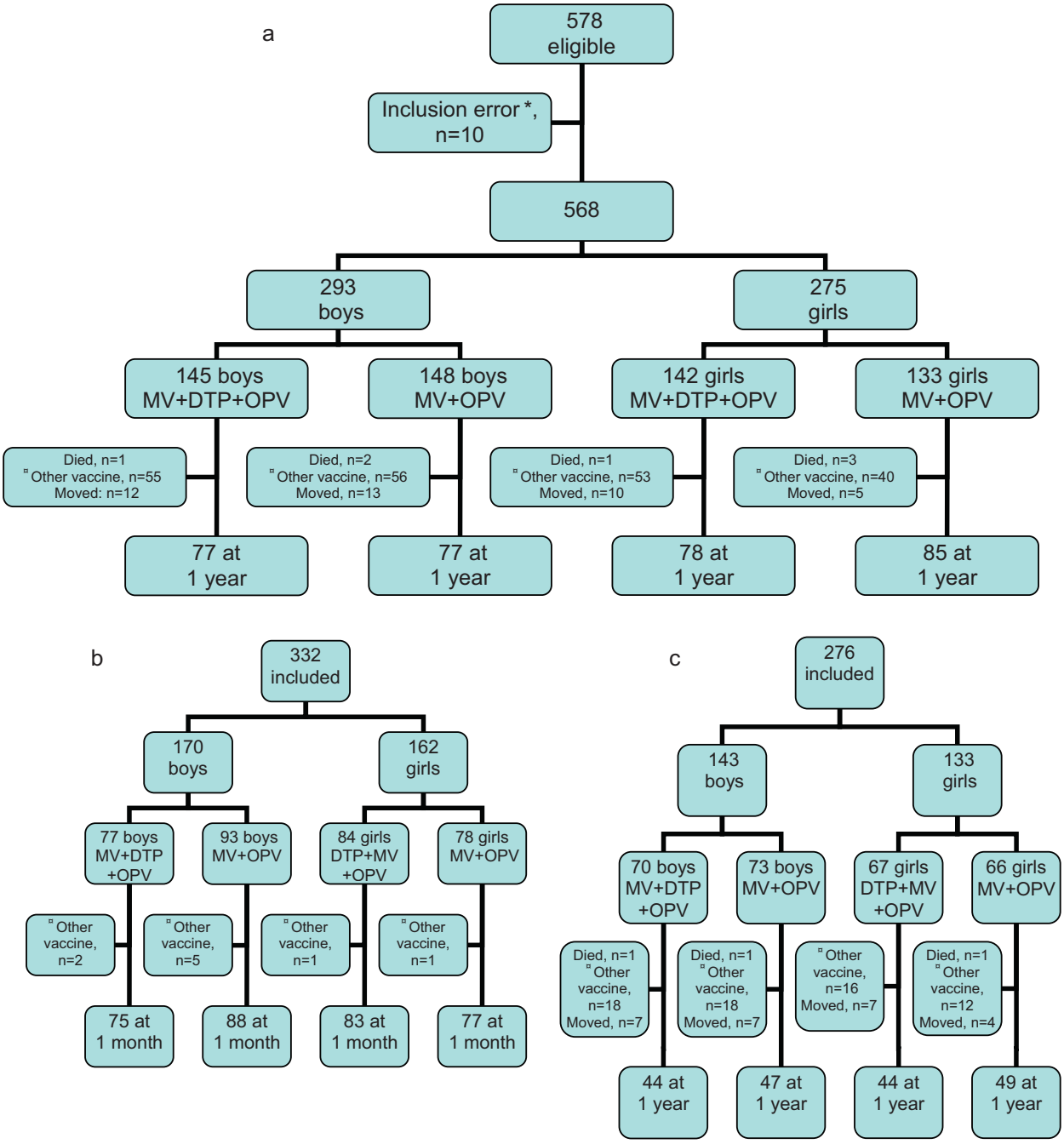


**Fig. 2.** Flow charts of: (a) total number of children eligible for MV + DTP + OPV group or MV + OPV group, (b) children included for adverse morbidity events sub-study, and (c) children included for anthropometry sub-study. *Wrong vaccine, wrong age, or did not live in study area. ¤ "Other vaccine" was (1) MV in the campaign 2006 after randomisation, (2) booster vaccination (children randomised for DTP3 + MV + OPV or MV + OPV where at 18 months of age provided DTP booster + OPV or OPV according to randomisation result), (3) DTP (+OPV) or MV provided at routine vaccination by mistake (few children). The majority of children in the sub-studies were included after May 2006 and therefore a smaller percentage of these children were lost due to "other vaccine". A total of 203 children were included in both sub-studies.

B vaccine + *H. influenza b* vaccine). When it became clear that we would never be able to complete the study as originally planned we decided to stop inclusions in April 2008, when only 568 had been randomised for the study. We had obtained data on adverse events in 332 children, anthropometric measurements in 276 children and followed the 568 children for specific diseases, consultations and hospitalisations for one year. Thus, although the study is incomplete, these data are reported in the present paper to encourage others to pursue similar studies.

### 2.8. Statistical methods

#### 2.8.1. Censoring

We excluded children who received another vaccine combination than the one they had been allocated, transpired to be older than 4 years of age, or to live outside the study area. Children were censored from follow-up if they received another vaccination (MV, DTP, or were earlier randomised for MV + DTP3 + OPV or MV + OPV and came for booster vaccination). Children enrolled in the study before 18 months of age kept their randomisation when they came

for booster vaccination, and were offered DTP booster + OPV if they received MV + DTP3 + OPV previously, and only OPV if they received MV + OPV. Both randomisation groups were censored from the analyses of the effect of DTP + MV + OPV vs. MV + OPV after booster vaccination. Children were also censored when they moved out of the study area or died.

#### 2.8.2. Background variables

Categorical variables were compared between randomisation groups using $\chi^2$-tests and continuous variables were analysed by *t*-tests.

#### 2.8.3. Morbidity

*Adverse morbidity events* were considered "present" when symptoms/signs following vaccination were registered one or more times throughout days 1–3 (*side effects*) or one or more times during days 7–31 (*late symptoms*) and analysed in binary regression models. The well known side effects are generally considered to be present during days 1–3 and no later than 7 days after DTP vaccination [13,14], and we evaluated late symptoms in the time period 7–31 days. "*Respiratory infection*" was defined as a maternal report of "running nose" and/or cough, "*Gastrointestinal infection*" as loss of appetite, vomiting and/or diarrhoea, and the inclusive "*Symptoms of morbidity*" was defined as presence of one or more of all the commonly occurring symptoms, loss of appetite, vomiting, diarrhoea, cough, running nose, fever, medication and/or consultation.

*Febrile diseases with vesicular rash* (*cambletch and varicella*), *consultations*, and *hospitalisations* were analysed as cumulative hazard ratios using Cox-regression. Failure was first case of cambletch or varicella, first consultation or first hospitalisation, respectively. Follow-up-time was one year. Children were censored when they experienced the illness, had another vaccination, moved out of the study area or died. One hundred and thirty eight (72 randomised to MV + DTP + OPV and 66 randomised to MV + OPV) children who had experienced "febrile disease with vesicular rash" at any time previous to randomisation were excluded in the analysis of febrile diseases with vesicular rash. This was done in order for children who were included in the analysis to be susceptible to chickenpox.

#### 2.8.4. Anthopometry

Anthropometric measures were analysed as *z*-scores based on data from the WHO Multicentre Growth Reference Study [15]. Assumptions of normality were checked by Q–Q plot and the distribution of residuals. Analyses were performed with and without outliers (2 children with impossible height-measurements at inclusion and 1 child with impossible MUAC at inclusion). Including the outliers had no impact on the results. Only results excluding outliers are presented.

#### 2.8.5. Adjustment

Adjustment was made one at a time in regression models (binary, Cox and linear respectively) for background variables differing between randomisation groups (Section 3.1) as well as for risk factors possibly affecting morbidity and growth (age, season, "symptoms of illness at inclusion") and for vaccination status at inclusion. Adverse morbidity events analyses were also run adjusted for the same symptom on the day of inclusion. In accordance with the randomised design, only unadjusted values are presented unless adjusted values provided additional information leading to other conclusions. Anthropometric *z*-scores were adjusted for age and sex, and using difference in *z*-scores in the regression model the estimates were also adjusted for *z*-score at inclusion.

#### 2.8.6. Stratification

All analyses were conducted for both sexes combined, adjusted for sex, as well as separately for boys and girls. Analyses of interaction between sex and DTP were performed in binary regression models in case of binary variables, in Cox regression models in case of hazard rates; and using likelihood ratio test for differences in anthropometric values. One hundred and one children had received MV before enrolment. Comparisons of the randomisation groups were therefore stratified in two groups: children who had received "MV *before* enrolment" and children who had "no MV before enrolment". MV before enrolment was received at a median of 152 days (IQR 84–279 days) before enrolment. Analyses were also performed stratified into groups of children randomised for MV + DTP3 + OPV or MV + OPV and children randomised for MV + DTP booster + OPV or MV + OPV.

#### 2.8.7. Female–male ratios

If we found significant sex-differential effects of the vaccinations we compared females and males within each the two treatment groups to investigate whether the difference was due to a beneficial effect of one of the treatments or a negative effect of the other treatment or both.

### 2.9. Ethical considerations

The mothers received written and oral information about the trial. It was explained that we do not know if it is better to give or not give DTP3/DTP booster vaccination with MV + OPV, and that participation was voluntary. The study was limited to children who had received at least two doses of DTP to ensure high protection against whooping cough (86% protection after the second dose vs. 96% after the third dose [16]) and tetanus [17]. The study was approved by The Danish National Committee on Biomedical Research Ethics and the Ministry of Health in Guinea-Bissau. The study was registered at Clinical trials.gov, number NCT00244673.

#### 2.9.1. Surveillance of pertussis vaccination and pertussis infection

At study start the coverage of DTP booster vaccination was estimated at 50% in the study area, assessed as the coverage in children 18–36 months of age whose vaccination card were seen at the three monthly visits by HDSS. Monthly calculations were made of the number of DTP vaccines used in the study area and this number did not drop during the study. From March 2007 DTP booster was no longer part of the routine vaccination schedule in Guinea-Bissau and booster vaccination was only provided to children participating in the present trial. When the children reached 4 years of age all children allocated to no DTP were offered DTP vaccination. Pertussis surveillance was performed one day every month at each health centre in the study area and at the hospital by sampling nasopharyngeal swabs from children 10 years or younger who came for consultation with a cough. There was no increase in the number of children with pertussis during the study.

## 3. Results

### 3.1. Background risk factors and loss to follow-up

Fig. 2 shows the trial profile for the total study and the two sub-cohorts. Background risk factors did not differ between randomisation groups, except for more medication in the MV + DTP + OPV group than in the MV + OPV group for all children and girls, and better toilet facilities for boys in the MV + DTP + OPV group than in the MV + OPV group (Appendix A). The median age at inclusion was 18.7 months (IQR 12.3; 19.9) and the mean weight at inclusion was 9.6 kg (95% CI 9.4; 9.7). In the sub-studies risk

factors which differed between randomisation groups were: "diarrhoea" (1% in the MV + DTP + OPV group vs. 7% in the MV + OPV group, $p = 0.02$) and "straw roof" (1% vs. 9%, $p = 0.02$) for girls in the adverse morbidity events sub-study; and "toilet facilities" (97% vs. 86% had toilet outside the house, $p = 0.02$) for girls in the anthropometry sub-study. We found no statistical significant difference in loss to follow-up in randomisation groups in the total cohort (boys: 47% in the MV + DTP + OPV group vs. 48% in the MV + OPV group, $p = 0.85$; and girls: 45% vs. 36%, $p = 0.13$ (Fig. 2(a))) or in the sub-studies (Fig. 2(b and c)).

The 287 children randomised for DTP3 (MV + DTP3 + OPV or MV + OPV) had a median age of 12.5 months (IQR 10.2; 18.5) and a mean weight of 9.0 kg (95% CI 8.8; 9.2); and 281 children randomised for DTP booster (MV + DTP booster + OPV or MV + OPV) had a median age of 19.4 months (IQR 18.7; 20.4) and a mean weight of 10.1 kg (95% CI 10.0; 10.3). Results were essentially the same in children randomised for DTP3 and in children randomised for DTP booster (data not shown) and in the following estimates are presented for the total group of 568 children. Stratification on MV or no MV *before* enrolment is presented for all outcomes.

### 3.2. Morbidity

#### 3.2.1. Adverse morbidity events

Well-documented *side effects* after DTP vaccination (reduced appetite, vomiting, crying, restlessness, fever, and local reaction at the vaccination site) [13,14] as well as "running nose" were significantly more common in the first 3 days following vaccination in the MV + DTP + OPV group compared with the MV + OPV group. We found a statistically significant interaction between sex and the effect of trial arm (DTP) for both diarrhoea and medication in the first three days after enrolment (Table 1). Thus, boys who received MV + DTP + OPV had borderline significant less diarrhoea than boys in the MV + OPV group (7% in the MV + DTP + OPV group vs. 18% in the MV + OPV group, $p = 0.05$) and the opposite tendency was seen for girls (19% in the MV + DTP + OPV group vs. 10% in the MV + OPV group, $p = 0.15$) ($p = 0.02$, test of interaction between DTP and sex) (Table 1). The female/male RR of diarrhoea was 2.70 (1.03; 7.09) in the MV + DTP + OPV group and 0.58 (0.26; 1.29) in the MV + OPV group. Furthermore, girls in the MV + DTP + OPV group had received medication 3.5 fold more often compared with the MV + OPV group, whereas there was virtually no increase for boys ($p = 0.02$, test of interaction between DTP and sex) (Table 1). The female/male RR of receiving medication was 1.54 (0.92; 2.55) in the MV + DTP + OPV group and 0.51 (0.24; 1.12) in the MV + OPV group. Adjustment did not change the estimates apart from the difference in diarrhoea prevalence becoming stronger after adjustment for diarrhoea at inclusion (adjusted $p = 0.003$, test for interaction between DTP and sex).

*Late symptoms* (during days 7–31 after vaccination) did not differ between the two randomisation groups, except for restlessness, which was less common in the MV + DTP + OPV group. There was a borderline significant ($p = 0.07$) interaction between sex and DTP on cough, though cough was not significantly more frequent in girls and less frequent in boys who received MV + OPV + DTP (Table 1).

#### 3.2.2. Febrile disease with vesicular rash

No cases of measles or whooping cough were registered at the three-monthly home visits. Of the 430 children who did not have *varicella* or *cambletch* before inclusion 36 reported "cambletch", 15 reported "varicella", and 43 reported cambletch and/or varicella over a 1 year follow-up time period. Both varicella and cambletch tended to be more common in the MV + DTP + OPV group compared with the MV + OPV group (hazard ratios (HR): 2.11 (0.72; 6.18) and 1.52 (0.77; 2.97), respectively). When the two outcomes were combined, the HR of "febrile disease with vesicular rash" was 1.86 (1.00; 3.47), 2.02 (0.86; 4.78) in girls and 1.57 (0.63; 3.94) in boys comparing the MV + DTP + OPV and MV + OPV groups (Fig. 3).

The higher risk of febrile diseases with vesicular rash in the MV + DTP + OPV group was only found among 356 children who had not received MV before enrolment (HR = 2.53 (1.20; 5.35)). The small group of 74 children who had received *MV before enrolment* had 11 cases of febrile disease with vesicular rash (HR was 0.80 (0.22; 2.99). Estimates did not differ by sex (data not shown).

#### 3.2.3. Medical consultations

One or more consultations were registered in 163 children of the 568 children during one year after enrolment, 85 (43 boys and 42 girls) in the MV + DTP + OPV group and 78 (40 boys and 38 girls) in the MV + OPV group. The HR for a first consultation in the year after enrolment for the MV + DTP + OPV group compared with the MV + OPV group was 1.16 (0.85; 1.57), the HR being 1.21 (0.78; 1.87) for boys and 1.11 (0.71; 1.72) for girls (Fig. 4). Adjusting HRs of febrile diseases and consultations for risk factors did not change the estimates.

The HR for consultations in the 101 children who had received *MV before enrolment* was 1.72 (0.73; 3.47) comparing the MV + DTP + OPV group (21 consultations in 52 children) with the MV + OPV group (13 in 49)); whereas there was no difference for the 467 children who had not received MV before enrolment (HR: 1.04 (0.73; 1.47) (64 of 235 vs. 65 of 232). Estimates in children who had and did not have MV before enrolment did not differ by sex (data not shown).

One hundred and forty two girls in the MV + DTP + OPV group had 6 consultations for diarrhoea whereas 133 girls in the MV + OPV

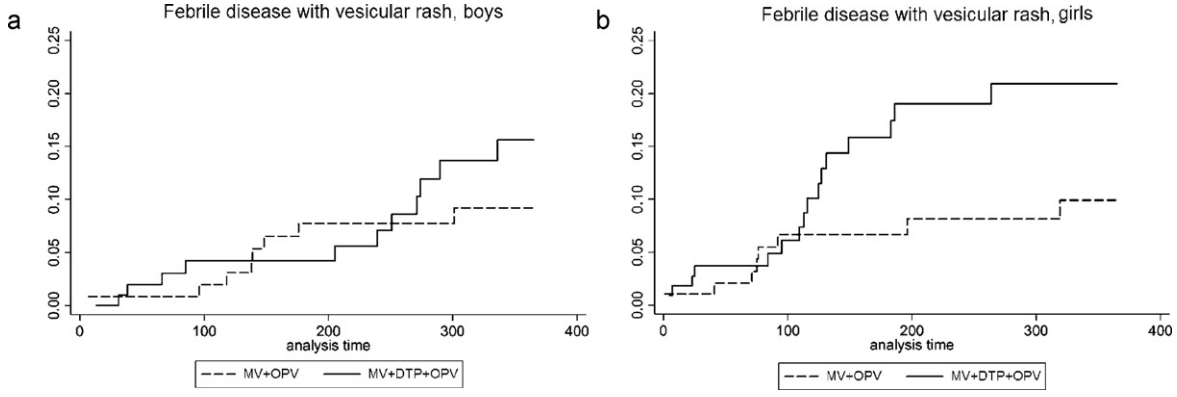


**Fig. 3.** Nelson–Aalen cumulative hazard estimate of "febrile disease with vesicular rash" during one year in the MV + DTP + OPV group and the MV + OPV group. (a) Boys and (b) girls. Percentage of children who had "febrile disease with vesicular rash" (*y*-axis) vs. time in days (*x*-axis). Solid line: DTP + MV + OPV. Dashed line: MV + OPV.

**Table 1**
Adverse events during one month after vaccination in MV + DTP + OPV group vs. MV + OPV group.

| | All | | | Boys | | | Girls | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | MV + DTP + OPV | MV + OPV | RR (95% CI)[a] | MV + DTP + OPV | MV + OPV | RR (95% CI) | MV + DTP + OPV | MV + OPV | RR (95% CI) | Interaction[*] |
| Days 1–3 | | | | | | | | | | |
| Crying | 83/154 | 18/169 | **5.11 (3.21; 8.12)** | 44/73 | 9/91 | **6.09 (3.18; 11.7)** | 39/81 | 9/78 | **4.17 (2.16; 8.05)** | 0.42 |
| Restlessness | 81/154 | 14/169 | **6.44 (3.81; 10.9)** | 43/73 | 8/91 | **6.70 (3.36; 13.4)** | 38/81 | 6/78 | **6.10 (2.73; 13.7)** | 0.86 |
| Subjective fever | 107/154 | 48/169 | **2.46 (1.89; 3.19)** | 49/73 | 30/91 | **2.04 (1.46; 2.85)** | 58/81 | 18/78 | **3.10 (2.02; 4.77)** | 0.13 |
| Loss of appetite | 21/154 | 6/169 | **3.83 (1.58; 9.28)** | 10/73 | 3/91 | **4.16 (1.18; 14.60)** | 11/81 | 3/78 | **3.53 (1.02; 12.2)** | 0.86 |
| Vomiting | 8/154 | 3/169 | 2.90 (0.79; 10.62) | 3/73 | 2/91 | 1.87 (0.32; 10.95) | 5/81 | 1/78 | 4.81 (0.57; 40.6) | 0.50 |
| Diarrhoea | 20/154 | 24/169 | 0.91 (0.53; 1.56) | 5/73 | 16/91 | 0.39 (0.15; 1.02) | 15/81 | 8/78 | 1.81 (0.81; 4.03) | **0.02** |
| Cough | 34/154 | 37/169 | 1.01 (0.67; 1.52) | 15/73 | 22/91 | 0.85 (0.48; 1.52) | 19/81 | 15/78 | 1.22 (0.67; 2.23) | 0.40 |
| Running nose[b] | 102/153 | 91/168 | **1.23 (1.03; 1.47)** | 48/72 | 51/90 | 1.18 (0.92; 1.50) | 54/81 | 40/78 | 1.30 (1.00; 1.70) | 0.59 |
| Respiratory rate > 40 cpm[b] | 13/152 | 13/166 | 0.95 (0.97; 1.03) | 5/72 | 8/89 | 1.01 (0.97; 1.06) | 8/80 | 5/77 | 0.98 (0.94; 1.03) | 0.37 |
| Temperature > 37 °C axillary[b] | 28/152 | 3/163 | **9.96 (3.08; 32.2)** | 11/72 | 1/89 | **13.6 (1.79; 103.5)** | 17/80 | 2/77 | **8.18 (1.95; 34.4)** | 0.69 |
| Medication | 46/152 | 26/168 | **1.95 (1.28; 2.98)** | 17/72 | 18/90 | 1.18 (0.66; 2.12) | 29/80 | 8/78 | **3.53 (1.72; 7.26)** | **0.02** |
| Days 7–31 | | | | | | | | | | |
| Crying | 5/151 | 12/156 | 0.43 (0.16; 1.19) | 2/72 | 7/82 | 0.33 (0.07; 1.52) | 3/79 | 5/74 | 0.56 (0.14; 2.28) | 0.61 |
| Restlessness | 3/151 | 12/156 | **0.27 (0.08; 0.92)** | 1/72 | 8/82 | 0.14 (0.02; 1.12) | 2/79 | 4/74 | 0.47 (0.09; 2.50) | 0.39 |
| Subjective fever | 35/151 | 47/156 | 0.78 (0.54; 1.14) | 20/72 | 29/82 | 0.80 (0.49; 1.26) | 15/79 | 18/74 | 0.78 (0.42; 1.44) | 0.99 |
| Loss of appetite | 5/151 | 7/156 | 0.77 (0.25; 2.39) | 4/72 | 5/82 | 0.91 (0.25; 3.28) | 1/79 | 2/74 | 0.47 (0.04; 5.10) | 0.63 |
| Vomiting | 4/151 | 7/156 | 0.63 (0.19; 2.13) | 4/72 | 6/82 | 0.76 (0.22; 2.59) | 0/79 | 1/74 | 0.00 (0.00-36.6) | – |
| Diarrhoea | 27/151 | 26/156 | 1.09 (0.67; 1.78) | 16/72 | 15/82 | 1.21 (0.65; 2.28) | 11/79 | 11/74 | 0.94 (0.43; 2.03) | 0.61 |
| Cough | 39/151 | 48/156 | 0.84 (0.59; 1.20) | 16/72 | 30/82 | 0.61 (0.36; 1.02) | 23/79 | 18/74 | 1.20 (0.70; 2.04) | 0.07 |
| Running nose[b] | 87/151 | 93/153 | 0.95 (0.79; 1.15) | 40/72 | 53/80 | 0.84 (0.65; 1.09) | 47/79 | 40/73 | 1.09 (0.82; 1.43) | 0.18 |
| Respiratory rate > 40 cpm[b] | 10/150 | 11/153 | 1.00 (0.97; 1.03) | 4/71 | 5/80 | 1.00 (0.96; 1.04) | 6/79 | 6/73 | 1.00 (0.96; 1.04) | 1.00 |
| Temperature > 37 °C axillary[b] | 7/150 | 5/153 | 1.48 (0.48; 4.59) | 5/71 | 3/80 | 1.88 (0.46; 7.61) | 2/79 | 2/73 | 0.92 (0.13; 6.43) | 0.56 |
| Medication | 22/151 | 26/153 | 0.88 (0.52; 1.47) | 13/72 | 17/80 | 0.85 (0.44; 1.63) | 9/79 | 9/73 | 0.92 (0.39; 2.21) | 0.88 |
| Gastrointestinal | 30/151 | 33/156 | 0.96 (0.62; 1.49) | 18/72 | 20/82 | 1.03 (0.59; 1.78) | 12/79 | 13/74 | 0.86 (0.42; 1.78) | 0.75 |
| Respiratory | 98/151 | 102/156 | 0.99 (0.84; 1.17) | 44/72 | 57/82 | 0.88 (0.70; 1.11) | 54/79 | 45/74 | 1.12 (0.89; 1.43) | 0.14 |
| Symptoms of morbidity | 100/151 | 108/156 | 0.96 (0.82; 1.12) | 47/72 | 62/82 | 0.86 (0.70; 1.06) | 53/79 | 46/74 | 1.08 (0.85; 1.37) | 0.17 |

Bold type indicates $p < 0.05$.

[a] Adjusted for sex.

[b] Children who were absent but with a mother or guardian at home had symptoms but not objective signs registered (number of children with registration of running nose, respiratory rate and temperature are smaller).

[*] $p$-Value for interaction between sex and DTP.

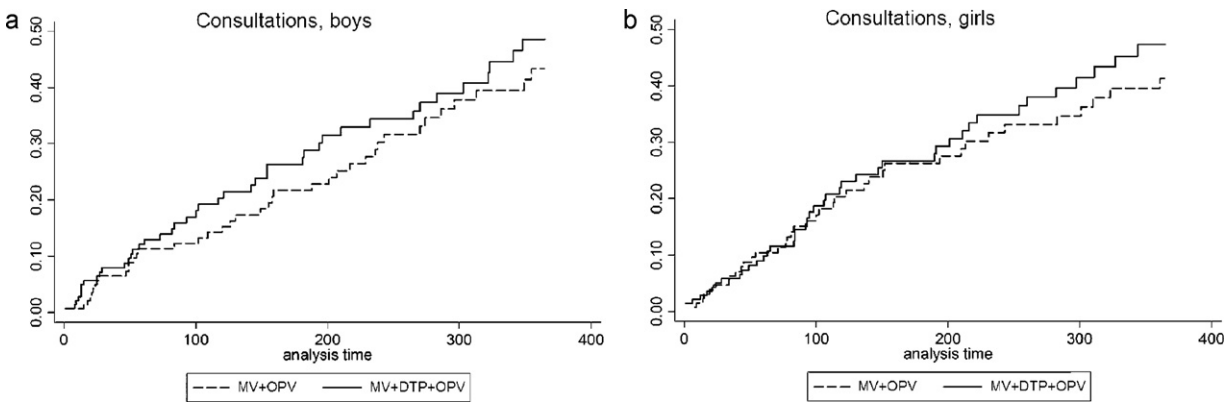


**Fig. 4.** Nelson–Aalen cumulative hazard estimate of consultation during one year in the MV + DTP + OPV group and the MV + OPV group. (a) Boys and (b) girls. Percentage of children who had one or more consultations (*y*-axis) vs. time in days (*x*-axis). Solid line: DTP + MV + OPV. Dashed line: MV + OPV.

group had only 1 consultation for diarrhoea (HR: 5.89 (0.71; 49.02)). The tendency was the opposite for boys (2 of 145 vs. 6 of 148, HR: 0.33 (0.07; 1.64)), ($p = 0.04$, test of interaction between sex and DTP). The female/male HR for consultation for diarrhoea was 2.98 (0.60; 14.9) in the MV + DTP + OPV group, and 0.16 (0.02; 1.36) in the MV + OPV group.

#### 3.2.4. Hospitalisations and mortality

Thirteen children (4 boys and 9 girls) with MV + DTP + OPV, and 9 children (5 boys and 4 girls) with MV + OPV as last vaccines, were hospitalised one or more times in the first year after enrolment. Comparing the MV + DTP + OPV group with the MV + OPV group, the HR for hospitalisation was 1.70 (0.70; 4.09); being 2.29 (0.71; 7.45) for girls and 0.96 (0.24; 3.85) for boys ($p = 0.40$ test for interaction between sex and DTP). The female/male HR for hospitalisation was 2.24 (0.69; 7.30) in the MV + DTP + OPV group, and 1.00 (0.25; 4.01) in the MV + OPV group. Mortality dropped in the study area during the study period and only 7 children died during the first year of follow up (Fig. 2(a)).

### 3.3. Growth

Anthropometric measures did not differ overall between the randomisation groups during follow-up (Table 2). However, results differed by sex. Boys were on average 1.1 kg heavier, 2 cm taller and had a 4 mm larger MUAC in the MV + DTP + OPV group than in the MV + OPV group at 9 months after enrolment; whereas girls weighed 1.0 kg less, were 1.2 cm shorter and had 4 mm smaller MUAC in the MV + DTP + OPV group than in the MV + OPV group at 9 months follow-up (Table 2). Thus, girls in the MV + DTP + OPV group had a consistent pattern of worse *z*-scores which was significant for weight-for-age *z*-score at 9 months and for the height-for-age *z*-score at 6 months (Table 2). The pattern was the opposite for boys, the interaction between DTP and sex being significant for weight at 6 and 9 months, for MUAC at 12 months and for weight-for-height at 6 and 9 months after enrolment (Table 2). Boys' weight-for-age and height-for-age *z*-scores in the anthropometry cohort differed between the two groups at enrolment (lower weight in the MV + OPV group) and we therefore adjusted for weight-for-age, height-for-age, MUAC-for-age and weight-for-height *z*-scores at enrolment in the analyses. Adjustment for risk factors did change a few of the estimates but not the overall conclusions (data not shown).

The sex-differential effect of DTP on growth was strongest for the 73 children who had received *MV before enrolment* (Appendix B).

Comparing girls and boys within the two randomisation groups, we found that girls grew significantly better than boys in the MV + OPV group and failed to grow compared with boys in the MV + DTP + OPV group at 12 months after randomisation, although this latter difference was not significant (Table 3).

## 4. Discussion

### 4.1. Main observations

Growth did not differ between randomisation groups. However, we found sex-differential effects of DTP combined with MV + OPV. Girls in the MV + DTP + OPV group grew less well compared with girls in the MV + OPV group, and the opposite tendency was seen in boys. There were also sex-differential effects of the vaccines on the adverse events diarrhoea and medication which were more common in girls in the MV + DTP + OPV group compared with the MV + OPV group, but not in boys. There were several indications that infections differed between the two randomisation groups; febrile diseases with vesicular rash being more frequent in the MV + DTP + OPV group compared with the MV + OPV group, and in girls all overall estimates of morbidity tended to show increased risk in the MV + DTP + OPV group.

### 4.2. Strengths and weaknesses

The non-specific effects of simultaneous MV and DTP (+OPV) vaccinations found in observational studies could have been due to confounding; for example, children coming late for vaccination and receiving MV and DTP simultaneously might have been frailer than children who had received their vaccinations on time, this frailty leading to increased morbidity and mortality. However, the results were the same in the present randomised study, and frailty cannot account for the opposite patterns emerging in boys and girls. Hence, confounding is unlikely to be the main cause of the observed negative effects of combined vaccinations.

In the present randomised study only 568 children were included in the analysis, and many children were lost during follow-up. Therefore we lost the power to show differences at a statistically significant level. Only symptoms day 1–3 and growth showed statistically significant differences and here the effect of MV + DTP + OPV was negative for girls. However, all overall estimates of morbidity were above 1 for girls, which strongly suggests, the effect of MV + DTP + OPV compared with MV + OPV on morbidity was negative in girls.

Children were censored due to the MV provided in the campaign after enrolment, and children who were randomised for MV + DTP3 + OPV or MV + OPV were censored when receiving booster vaccination at 18 months or later. Furthermore, in Bissau there is a large migration in and out of the area. Thus, it is not

**Table 2**
Difference between randomisation groups' average weight-for-age *z*-score, height-for-age-*z*-score, mid-upper-arm-circumference-for-age *z*-score, and weight-for-height *z*-score at 3, 6, 9, and 12 months after randomisation to the MV + DTP + OPV group or the MV + OPV group.

| Anthropometric measures at time[¤] after enrolment | All[a] | | | Boys | | | Girls | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean (sd) MV + DTP + OPV | Mean (sd) MV + OPV | *z*-score in MV + DTP + OPV minus *z*-score in MV + OPV group[b] | Mean (sd) MV + DTP + OPV | Mean (sd) MV+OPV | *z*-score in MV + DTP + OPV minus *z*-score in MV + OPV group[b] | Mean (sd) MV + DTP +OPV | Mean (sd) MV +OPV | *z*-score in MV + DTP + OPV minus *z*-score in MV + OPV group[b] | Interaction[c] |
| Weight 0 in kg, *n* = 276 | 9.5 (1.5) | 9.4 (1.5) | | 9.9 (1.3) | 9.5 (1.7) | | 9.1 (1.5) | 9.2 (1.4) | | |
| Weight 3 in kg, *n* = 183 | 10.0 (1.6) | 9.9 (1.6) | −0.05 (−0.22;0.12) | 10.4 (1.6) | 9.8 (1.6) | 0.08 (−0.19;0.36) | 9.7 (1.5) | 10.0 (1.6) | −0.18 (−0.36;0.01) | 0.16 |
| Weight 6 in kg, *n* = 149 | 10.7 (1.7) | 10.7 (1.7) | 0.02 (−0.16;0.19) | 11.2 (1.5) | 10.7 (1.7) | **0.24 (0.02;0.47)** | 10.1 (1.7) | 10.7 (1.6) | −0.23 (−0.50;0.05) | **0.02** |
| Weight 9 in kg, *n* = 125 | 11.4 (1.9) | 11.2 (1.5) | 0.01 (−0.23;0.22) | 11.9 (1.6) | 10.8 (1.5) | 0.29 (−0.02; 0.60) | 10.7 (2.0) | 11.7 (1.4) | **−0.33 (−0.65;−0.02)** | **0.01** |
| Weight 12 in kg, *n* = 104 | 12.2 (1.7) | 11.8 (1.6) | 0.03 (−0.19;0.26) | 12.6 (1.6) | 11.6 (1.6) | 0.24 (−0.07;0.54) | 11.8 (1.7) | 12.0 (1.5) | −0.20 (−0.54;0.14) | 0.06 |
| Height 0 in cm, *n* = 271 | 77.8 (5.7) | 77.3 (5.5) | | 79.1 (5.4) | 77.3 (5.4) | | 76.4 (5.8) | 77.2 (5.7) | | |
| Height 3 in cm, *n* = 179 | 78.1 (5.7) | 78.2 (5.4) | 0.01 (−0.17;0.20) | 78.4 (5.3) | 78.0 (5.2) | −0.02 (−0.26;0.22) | 77.8 (6.2) | 78.4 (5.8) | 0.05 (−0.24;0.33) | 0.70 |
| Height 6 in cm, *n* = 145 | 80.6 (5.9) | 81.4 (5.0) | −0.20 (−0.41;0.01) | 81.9 (5.4) | 81.0 (4.8) | −0.02 (−0.30;0.26) | 79.4 (6.2) | 82.0 (5.2) | **−0.40 (−0.72;−0.07)** | 0.12 |
| Height 9 in cm, *n* = 121 | 83.6 (6.0) | 83.6 (5.0) | −0.18 (−0.46;0.11) | 84.5 (5.6) | 82.5 (4.8) | −0.09 (−0.50;0.32) | 82.6 (6.5) | 84.8 (5.2) | −0.27 (−0.66;0.13) | 0.68 |
| Height 12 in cm, *n* = 100 | 87.4 (4.0) | 86.3 (5.1) | −0.05 (−0.33;0.24) | 88.4 (3.9) | 85.3 (4.2) | 0.21(−0.19,0.61) | 86.2 (4.0) | 87.3 (4.2) | −0.34 (−0.76;0.08) | 0.07 |
| MUAC 0 in mm, *n* = 275 | 142 (13) | 141 (12) | | 145 (12) | 142 (12) | | 139 (13) | 140 (12) | | |
| MUAC 3 in mm, *n* = 182 | 148 (11) | 146 (11) | 0.02 (−0.19;0.23) | 149 (10) | 146 (12) | 0.14 (−0.19;0.48) | 146 (11) | 146 (10) | −0.11 (−0.37;0.16) | 0.33 |
| MUAC 6 in mm, *n* = 148 | 149 (11) | 150 (10) | −0.07 (−0.27;0.13) | 152 (10) | 150 (11) | 0.02 (−0.27;0.30) | 147 (12) | 149 (10) | −0.17 (−0.44;0.10) | 0.45 |
| MUAC 9 in mm, *n* = 124 | 151 (12) | 151 (11) | −0.02 (−0.23;0.20) | 154 (10) | 150 (12) | 0.10 (−0.20;0.40) | 148 (13) | 152 (9) | −0.16 (−0.47;0.16) | 0.26 |
| MUAC 12 in mm, *n* = 104 | 156 (10) | 154 (10) | −0.01 (−0.23;0.22) | 158 (9) | 153 (11) | 0.23 (−0.07;0.54) | 153 (11) | 155 (10) | −0.30 (−0.63;0.03) | 0.03 |
| Weight-for-height 3 | | | 0.02 (−0.23; 0.26) | | | 0.29 (−0.07; 0.66) | | | −0.26 (−0.58; 0.06) | **0.02** |
| Weight-for-height 6 | | | 0.18 (−0.07; 0.43) | | | **0.36 (0.05; 0.67)** | | | −0.02 (−0.43; 0.39) | 0.16 |
| Weight-for-height 9 | | | 0.11 (−0.21; 0.43) | | | **0.51 (0.07; 0.95)** | | | −0.37 (−0.82; 0.09) | **<0.01** |
| Weight-for-height 12 | | | 0.15 (−0.15; 0.45) | | | 0.24 (−0.14; 0.63) | | | −0.04 (−0.46; 0.53) | 0.51 |

*n*, number of children in analysis (measured, and not censored or outliers).

[¤] Baseline 3, 6, 9, and 12 months after enrolment.

[a] Adjusted for sex.

[b] In the regression model of difference in weight-for-age, height-for-age, MUAC-for-age and weight-for-length *z*-score analyses were adjusted for measure obtained on the day of enrolment and age, (): 95% confidence interval.

[c] Interaction between sex and DTP. Bold type: $p < 0.05$.

**Table 3**
Relative female and male growth in the two randomisation groups.

| | All children[a] | MV + DTP + OPV group | MV + OPV group |
|---|---|---|---|
| Measure at 12 months fu | Difference in *z*-scores at 12 month follow-up girls minus boys[b] | | |
| Weight-for-age, *n* = 104 | 0.09 (−0.13; 0.32) | −0.15 (−0.54; 0.23) | **0.32 (0.06; 0.59)** |
| Height for age, *n* = 100 | 0.20 (−0.08; 0.49) | −0.09 (−0.47; 0.28) | 0.41 (−0.03; 0.84) |
| MUAC-for age, *n* = 104 | −0.04 (−0.26; 0.18) | −0.32 (−0.67; 0.02) | 0.17 (−0.11; 0.46) |
| Weight-for-height, *n* = 104 | 0.05 (−0.25; 0.35) | −0.07 (−0.56; 0.42) | 0.15 (−0.24; 0.54) |

Bold type: $p < 0.05$.
[a] Adjusted for randomisation group.
[b] Adjusted for inclusion measure.

surprising that a large number of the children had their follow-up censored within one year. Children travelling with their parents (often due to lack of permanent jobs) may have had worse health conditions. In contrast, children who stayed in the study area, and were censored because they were brought for another vaccination after enrolment, may have had better health conditions. Hence, the losses went in two directions. Further, losses did not differ by randomisation group. With the randomised design there is little reason to believe that the present comparisons have been biased.

Looking at background risk factors in the total group of 568 children, the health at enrolment may have been worse in girls (more medication) in the MV + DTP + OPV group than in the MV + OPV group. However, in the sub-studies girls had better health at enrolment (less diarrhoea, fewer straw roofs) in the MV + DTP + OPV group than in the MV + OPV group. We evaluated that the differences found in some of the more than 20 tested background risk factors (in each sex and in both sub-studies) could be explained by chance. Adjustment for background factors differing between randomisation groups did not change the estimates. We presented unadjusted estimates throughout the paper (except for the difference in *z*-scores which are adjusted for inclusion value and age per se) in order to keep the random distribution between vaccination groups.

For logistic reasons the subgroup studies did not include all children enrolled in the trial and this selection process could also have created some imbalances between the groups. Despite this the randomised design should have limited bias and confounding.

The diagnoses in health centres and at the main hospital in Bissau are mainly clinical and we are not able to categorise into pathogen-specific diagnoses. Five percent of the children had one or more hospitalisations during a one year time period. The average annual hospitalisation rate in the study area was 7% in previous studies [18].

Due to the measles vaccination campaign for all children between 6 months and 15 years of age, some children had received MV in a campaign before being randomised. Since campaign measles vaccinations are not considered to replace the routine MV the children would still receive MV when brought for routine vaccination and thus were still eligible to receive both MV and DTP. This plan to carry out a national MV campaign was not known when the present study was initiated. We would have preferred to have only children who had followed the official vaccination schedule. However, other interventions and campaigns are very common and may affect the results. We therefore took the inclusion of children who had received an additional MV in campaign as a "natural experiment". We stratified the analyses in children who received and children who did not receive MV before enrolment. It varied whether the trend was strongest in one or the other subgroup. Studies of the non-specific effects of vaccines have essentially only dealt with the first dose of MV [1]. The fact that the sex-differential effect of DTP on growth was particularly strong in the group which had received MV before enrolment suggests that two doses of MV may modify the non-specific effects and interact with DTP, although this was not statistically significant in the present study. Some of the stratified groups were small and some insignificant results may have been false. On the other hand, evaluation of both sexes in different strata produced many estimates, some of which may have shown statistical difference by chance. Thus, conclusions in the stratified analyses should be interpreted with caution.

Children who received MV in the campaign also received vitamin A. Vitamin A has been shown to interact with DTP [19,20] and might also be responsible for an interaction between "participation in the campaign" and MV + DTP + OPV. As children were not randomised for MV in the campaign before enrolment other unknown confounders may also be responsible for differences in children who had MV before enrolment and children who did not.

There were major changes in vaccination coverage during the present study and mortality was much lower (7/568, 1.2%) than originally anticipated (3%). Furthermore, Guinea-Bissau decided to change from DTP to pentavalent vaccine while the study was ongoing. We were therefore unable to complete the study and measure mortality as originally planned. However, there is no reason to believe that this would make the results for growth and morbidity non-representative.

Wasting (weight-for-height *z*-score below −2) as well as underweight (weight-for-age *z*-score below −2) is associated with morbidity and mortality [21,22]. Mild underweight is very common in low-income countries and account for a large total burden of disease [23]. Stunting (height-for-age *z*-score below −2) is caused by long-term insufficient nutrient intake and frequent infections [22]. Therefore, we chose to present weight-for-age, along with the other measures height and MUAC, as well as weight-for-height. In girls, all measures tended to be negatively affected by MV + DTP + OPV compared with MV + OPV.

Boys in the MV + OPV group had lower *z*-scores at enrolment, and this was adjusted for in the analyses of differences between measures at enrolment and measures at follow-up. Boys with low weight at enrolment may have a better growth potential, but may also continue not to grow. With the randomised design we assume the growth potential was the same in the 2 groups of boys. However, morbidity measures did not show a common trend for boys, and this randomised trial does not enable us to conclude whether MV + DTP + OPV had an effect on morbidity in boys.

### 4.3. Interpretation

Children who experience repeated infections fail to thrive, and the burden of infectious diseases may be assessed by looking at growth. Infectious diseases are the main cause of child death in low-income countries and growth may be one of the best markers for risk of subsequent mortality [21]. The strongest finding in the present study was therefore undoubtedly the difference in growth for girls. The differences in weight-for-age *z*-scores suggest that

the tendency for better growth in boys and worse in girls in the MV + DTP + OPV group compared with the MV + OPV group were present from 3 months of age, and became significant 6 months after vaccination for boys and 9 months after vaccination for girls. Only girls were differently affected by the vaccines to an extent that affected height. The biological explanation might be repeated infections in the 6–9 month after vaccination. These infections were not lethal, but the results may indicate that repeated infections and the concomitant impairment of growth could lead to death in girls with DTP (as their most recent vaccination) in situations with higher child mortality [4,5].

The results on infections (morbidity) were consistent with the negative effect of MV + DTP + OPV on growth relative to MV + OPV in girls.

The sex-differential results could be interpreted in two ways; the combination of DTP + MV + OPV might have deleterious non-specific effects for girls, or MV + OPV might be beneficial for girls but the effect lost when MV + DTP + OPV are given simultaneously. When MV + DTP + OPV was associated with impaired outcomes in girls only, we consistently found a female-male ratio below 1 in the MV + OPV group and a female–male ratio above 1 in the MV + DTP + OPV group. A reduced female–male mortality ratio among measles-vaccinated children has been observed consistently in previous observational studies [1,4,5,9–11]. We have also observed consistently that DTP is associated with female–male mortality ratios above 1 [3–6,9–11]. Further, the harmful effect of DTP for girls seems to be present when DTP, as usual practice, is provided with OPV but also when OPV was not available and DTP is provided alone [7]. The present study suggests that the combination of MV + DTP + OPV may have a negative effect for girls. However, it should be noted that in previous studies, in which the controls were children who had received MV only, we found a negative effect of MV + DTP + OPV in both sexes [8,9]. In the present study, in which the controls had received both MV and OPV, there was no negative effect of MV + DTP + OPV for boys. We have reported that BCG and OPV administered simultaneously had a negative effect for boys [24]. Nothing is known about whether MV and OPV administered simultaneously have any particular effects in boys; but if it does this might have influenced the assessment of the effect of MV + DTP + OPV for boys in the present trial.

Sex differences in susceptibility to many infections have been described [25–27] and inflammatory markers may differ in boys and girls during infection [28], but there are only limited data showing that males and females respond differently to vaccinations [29]. Sex hormone levels are quite different in males and females in the first few years of life [30] and many immune cells have sex hormone receptors providing a mechanism whereby sex differences in the adaptive immune response to vaccination might arise. Sex hormones are able to manipulate the innate immune system and could thus influence the innate immune response to vaccination [31]. Sex differences could also be due to the numerous X-chromosome immune response genes which are subject to inactivation or silencing of one of the two alleles in females, while males only have one copy of each gene on their single X chromosome [32]. No immunological study has been published of the possible differential effect of administering MV + DTP + OPV compared with MV + OPV, and why these effects differ for boys and girls. Such detailed immunological studies will clearly need to be carried out to fully understand the underlying mechanisms of vaccine interactions and sex differences, and such studies are currently ongoing in The Gambia.

### 4.4. Consistency with previous findings

We found side effects of DTP consistent with earlier studies [13,14]. More colds ("running nose") were registered 1–3 days after vaccination in the MV + DTP + OPV group, although "colds" have not previously been described as an adverse effect of DTP. Girls more often received medication on days 1–3 after vaccination than boys. Gender differences in the use of medication were not found in studies reporting use of non-prescribed drugs in Africa [33,34] and more medication for girls most likely reflects that girls were sicker than boys.

Better growth in girls in the MV + OPV group is in agreement with findings in earlier observational studies of a potential negative effect of combined DTP (+OPV) and MV [8–11], but also the studies showing that MV is associated with more beneficial effects for girls than for boys [3–5,9,11]. Whether or not all episodes of "febrile disease with vesicular rash" represent chickenpox [12], it is intriguing that infections easily recognized by the mother tended to differ between randomisation groups. Diarrhoea registered at adverse events follow-up and at consultations was more common in girls and less common in boys in the MV + DTP + OPV group compared with the MV + OPV group. These findings are consistent with previous studies showing DTP + OPV to be associated with increased incidence of specific diarrhoeal pathogens for girls [35,36].

### 4.5. Conclusions and implications

This is the first randomised trial of non-specific effects of DTP. Consistent with previous observational studies [8–11], the present randomised trial indicates that the current practice of combining DTP + OPV with MV had negative effects on growth and morbidity for girls. If combined vaccinations have negative effects, the current recommendation to administer missing vaccines simultaneously should be reconsidered. This may be particularly important because the international health community is measuring the performance of the vaccination programme through the coverage for DTP3, and there is therefore a drive to increase the coverage for DTP vaccinations [37,38]. In many rural areas a large proportion of the children are receiving MV and DTP + OPV simultaneously; for example, in rural Guinea-Bissau nearly one-third of the children receives the two vaccines together.

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## Appendix A. Background factors in children randomised for the MV + DTP + OPV group or the MV + OPV group, *N* = 568.

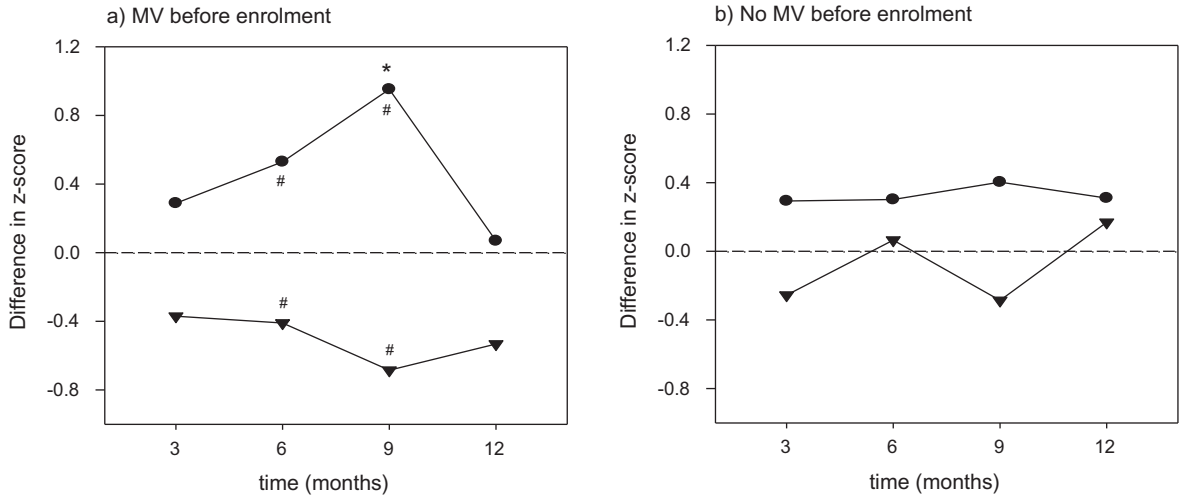
| | All | | Boys | | Girls | |
|---|---|---|---|---|---|---|
| | MV + DTP + OPV group *n* (%) | MV + OPV group *n* (%) | MV + DTP + OPV group *n* (%) | MV + OPV group *n* (%) | MV + DTP + OPV group *n* (%) | MV + OPV group *n* (%) |
| Number of children | 287 | 281 | 145 | 148 | 142 | 133 |
| Included at health centre in | | | | | | |
| Bandim district | 138 (48) | 130 (46) | 69 (48) | 65 (44) | 69 (49) | 65 (49) |
| Belem district | 47 (16) | 47 (17) | 28 (19) | 25 (17) | 19 (13) | 22 (17) |
| Cuntum district | 102 (36) | 104 (37) | 48 (33) | 58 (39) | 54 (38) | 46 (35) |
| Included | | | | | | |
| At routine vaccination | 58 (20) | 49 (17) | 29 (20) | 24 (16) | 29 (20) | 25 (19) |
| Found in BHP database | 229 (80) | 232 (83) | 116 (80) | 124 (84) | 113 (80) | 108 (81) |
| Included in rainy season[a] | 129 (45) | 133 (47) | 64 (44) | 69 (47) | 65 (46) | 64 (48) |
| Ethnicity | | | | | | |
| Pepel | 103 (36) | 91 (32) | 50 (34) | 44 (30) | 53 (37) | 47 (35) |
| Fula | 75 (26) | 76 (27) | 41 (28) | 39 (26) | 34 (24) | 37 (28) |
| Other | 109 (38) | 114 (41) | 54 (37) | 65 (44) | 55 (39) | 49 (37) |
| Mother 4 years of schooling or less 70[b] | 163 (64) | 163 (67) | 83 (65) | 84 (65) | 80 (63) | 79 (70) |
| 3 siblings or more 12[b] | 74 (26) | 77 (28) | 37 (26) | 40 (28) | 37 (26) | 37 (28) |
| Straw roof 12[b] | 12 (4) | 17 (6) | 9 (6) | 8 (6) | 3 (2) | 9 (7) |
| No electricity in house 12[b] | 227 (81) | 223 (81) | 111 (78) | 112 (78) | 116 (83) | 111 (85) |
| No television 13[b] | 234 (83) | 226 (83) | 120 (85) | 116 (81) | 114 (81) | 110 (85) |
| Toilet outside the house 13[b] | 251 (89) | 254 (93) | **121 (86)** | **137 (95)** | 130 (93) | 117 (90) |
| Medical history at inclusion | | | | | | |
| Not breastfeeding 5[b] | 79 (28) | 74 (26) | 44 (30) | 38 (26) | 35 (25) | 36 (27) |
| Measles infection 9[b] | 5 (2) | 6 (2) | 4 (3) | 4 (3) | 1 (1) | 2 (2) |
| Previously hospitalised 9[b] | 27 (10) | 30 (11) | 13 (9) | 18 (12) | 14 (10) | 12 (9) |
| TB case in the house 16[b] | 10 (4) | 11 (4) | 6 (4) | 8 (6) | 4 (3) | 3 (2) |
| On the day of inclusion | | | | | | |
| Had medicine 2[b] | **25 (9)** | **12 (4)** | 12 (8) | 8 (5) | **13 (9)** | **4 (3)** |
| Symptoms | | | | | | |
| Cough | 54 (19) | 49 (17) | 22 (15) | 27 (18) | 32 (23) | 22 (17) |
| Vomit 2[b] | 6 (2) | 4 (1) | 5 (3) | 1 (1) | 1 (1) | 3 (2) |
| Diarrhoea | 25 (9) | 27 (10) | 17 (12) | 15 (10) | 8 (6) | 12 (9) |
| Fever 43[b] | 22 (8) | 20 (8) | 6 (4) | 10 (7) | 16 (12) | 10 (8) |
| Respiratory count >40 cpm 108[b] | 24 (11) | 22 (9) | 14 (12) | 16 (13) | 10 (9) | 6 (6) |
| Temperature > 37 °C | 7 (2) | 7 (2) | 1 (1) | 2 (1) | 6 (4) | 5 (4) |
| Objective signs of infection | 28 (12) | 30 (13) | 16 (14) | 18 (15) | 12 (11) | 12 (11) |
| | Mean (sd) | Mean (sd) | Mean (sd) | Mean (sd) | Mean (sd) | Mean (sd) |
| Age (mo) | 17.6 (5.8) | 17.3 (5.7) | 17.9 (5.5) | 17.3 (6.0) | 17.4 (6.0) | 17.3 (5.3) |
| ±DTP3 | 14.9 (6.0) | 14.2 (4.9) | 15.1 (5.8) | 14.2 (5.1) | 14.7 (6.1) | 14.2 (4.7) |
| ±DTP-booster | 20.3 (4.1) | 20.6 (4.5) | 20.4 (3.7) | 20.8 (5.0) | 20.2 (4.5) | 20.4 (4.0) |
| Maternal MUAC 73[b] | 270 (33) | 267 (34) | 272 (32) | 267 (28) | 268 (34) | 266 (39) |
| Weight (kg) 1[b] | 9.6 (1.7) | 9.5 (1.6) | 10.0 (1.7) | 9.7 (1.6) | 9.2 (1.6) | 9.3 (1.6) |
| Height (cm) 4[b] | 78.2 (6.0) | 77.8 (5.5) | 79.1 (5.7) | 78.0 (5.5) | 77.3 (6.2) | 77.6 (5.6) |
| MUAC (mm) | 143 (14) | 142 (12) | 146 (15) | 143 (12) | 141 (13) | 141 (13) |
| Size of BCG-scar (mm) 107[b] | 3.7 (2.1) | 3.5 (2.1) | 3.7 (2.1) | 3.7 (2.1) | 3.8 (2.1) | 3.4 (2.0) |

Bold type: differ in randomisation groups ($p < 0.05$).

[a] Rainy season in Guinea-Bissau is from May to October. cpm: counts per minute.

[b] Missing information. Respiratory count, objective signs and BCG diameter were only collected from children found in the registration-database.

**Appendix B. Differences in growth (weight-for-height *z*-score in MV + DTP + OPV group minus weight-for-height *z*-score in MV + OPV group, adjusted for inclusion value), stratified by MV or no MV before enrolment in the study. (a) MV before enrolment and (b) no MV before enrolment.**




Positive values indicate larger *z*-score in children in the MV + DTP + OPV group compared with the MV + OPV group. Circle: weight-for-height *z*-score for boys. Triangle: weight-for-height *z*-score for girls. *Significant difference between MV + DTP + OPV group and MV + OPV group. #*P*-value < 0.05 for interaction between sex and DTP. *P*-values for interaction between "MV before enrolment" and DTP were all above 0.05 (in boys and in girls).

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